DTPM COVID-19 RT-PCR Test

Instructions for Use

For Use Under Emergency Use Authorization (EUA) Only

Rx Only

For in vitro diagnostic use

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Version 1.0

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1.0 Intended Use

The DTPM COVID-19 RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasal aspirate, and nasal wash specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasal aspirate, and nasal wash specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definitive cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

The DTPM COVID-19 RT-PCR test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The DTPM COVID-19 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

2.0 Explanation of Test

The DTPM COVID-19 RT-PCR test is a reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of human SARS-CoV-2 RNA in nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, and nasal washes. The test utilizes one primer and probe set to detect a conserved region in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect a human S9 ribosomal gene in a clinical sample. RNA isolated from specimens is reverse transcribed to cDNA and subsequently amplified using a ThermoFisher QuantStudio 5 instrument with software version 1.5.1. During the amplification process, the probe anneals to a specific target sequence between the forward and reverse primers. The 5' exonuclease activity of Taq polymerase degrades the

bound probe during the extension phase of the PCR cycle, which causes the 5' labeled reporter dye to separate from the 3' nonfluorescent quencher (NFQ), generating a fluorescent signal. During PCR amplification, fluorescence generated by degradation of the target-specific probe is monitored by the QuantStudio 5 RT-PCR instrument.

Selective amplification of target nucleic acid from the sample is achieved by the use of target specific forward and reverse primers and probe (FAM) specific to conserved regions of the N genes for SARS-CoV-2.

Selective amplification of Endogenous Control is achieved by the use of non-competitive, sequence specific forward and reverse primers and a probe (VIC) which have no homology with the coronavirus genome.

2.1 Materials Provided

Kit Content:

1. DTPM COVID-19 RT-PCR Multiplex Assay Mix
2. ThermoFisher Fast Virus 1-Step Enzyme Master Mix
3. DTPM COVID-19 RT-PCR Multiplex Assay Positive Control Mix

2.2 Additional Materials Required but Not Provided

Extraction Kits:

The following nucleic acid extraction kits are validated for use with this test:

- Qiagen QIAamp Viral RNA Kit, Cat. No. 52906
- Qiagen QIAamp 96 Viral RNA Kit, Cat. No. 52962
- Indical Biosciences IndiMag Pathogen Kit, Cat. No. SP947457
- Omega Biotek Mag-Bind Viral DNA/RNA 96 Kit, Cat. No. M6246-02
- ThermoFisher MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit, Cat. No. A48383

Instruments:

The following PCR instruments are validated for use with this test:

- ThermoFisher QuantStudio 5
- ThermoFisher KingFisher Flex

Materials Required but not provided:

▪ Disposable Sampling Tube – MTM (15º C – 25º C)	DTPM	Cat# DTPM-MTM
▪ Molecular Grade Water (0º C – 40º C)	Fisher	Cat# SH30538.02
▪ Ethanol 200 proof (100%) (0º C – 40º C)	Fisher	Cat# 04-355-450
▪ Isopropyl Alcohol (0º C – 40º C)	Fisher	Cat# MPX18341

▪ Phosphate Buffered Saline (PBS) (15º C – 30º C)	Fisher	Cat# 70011044
▪ RNAse Away	Fisher	Cat# 21-402-178
▪ 1.5 mL Microcentrifuge Tubes	VWR	Cat# 1002-726
▪ 2.0 mL Screw Cap Microcentrifuge Tube	VWR	Cat# 16466-058
▪ Pipette Tips, filtered, sterile, various sizes		
▪ 384 Well PCR Plates	VWR	Cat# 60941-078
▪ 2.2 mL 96-well Deep Well Plates	VWR	Cat# 76329-996
▪ 0.5 mL 96-well Elution Plate	VWR	Cat# 76210-518
▪ Optical Adhesive PCR Film	ThermoFisher	Cat# 4360954
▪ Kim-Wipes™ tissues	VWR	Cat# 470224-038
▪ Multichannel Pipette	ThermoFisher	Cat# 4672070BT
▪ Vortex-Genie® 1 Touch Mixer	VWR	Cat# 14216-184
▪ Centrifuge (Microfuge mySPIN™6)	ThermoFisher	Cat# 75004061
▪ 96-well microtube racks	VWR	Cat# 21150-234

2.3 Reagent Storage

- Store all kit components at -20ºC until ready for use.
- Follow manufacturer's guidelines for storage requirements for additional components.
- Do not use if damaged.
- Store reagents separate from sample materials to avoid contamination.

3.0 Specimen Storage and Handling

3.1 Specimen

- The DTPM COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of human SARS-CoV-2 RNA in ***nasopharyngeal (NP) swab, oropharyngeal (OP) swab, anterior nasal swab, mid-turbinate nasal swab, nasal aspirate, and nasal wash specimens***.

- Collect samples using only sterile, flocked, nylon swabs with plastic shafts. DO NOT use calcium alginate swabs or swabs with wooden shafts as they *may* contain substances which degrade some viruses and inhibit some molecular assays.

3.2 Specimen – Storage and Handling

- Store respiratory specimens at 2-8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or below.
- Store extracted nucleic acid samples at -70°C or lower.
- Treat all clinical specimen as potentially infectious materials.

4.0 Warnings

4.1 Warnings and Precautions

4.1.1 For in vitro diagnostic use
4.1.2 Rx only
4.1.3 For use under Emergency Use Authorization (EUA) only
4.1.4 This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
4.1.5 This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
4.1.6 The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
4.1.7 This product is for use in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
4.1.8 Follow Centers for Disease Control and Prevention (CDC) recommendations for safe handling of potentially infectious samples.
4.1.9 Wear appropriate Personal Protective Equipment (PPE) when handling reagents.
4.1.10 Do not use expired reagents and/or materials.
4.1.11 DO NOT use calcium alginate swabs or swabs with wooden shafts as they may contain substances which degrade some viruses and inhibit some molecular assays.
4.1.12 **Do not use bleach to clean areas exposed to guanidine thiocyanate**. There is a risk of exposure to harmful cyanide gas, a by-product of a reaction between guanidine thiocyanate or similar chemicals and bleach (sodium hypochlorite), when certain transport media are used with an incompatible testing platform or laboratory process. Guanidine thiocyanate may be referred to as guanidinium rhodanide, guanidinium thiocyanate, or guanidinium.
4.1.13 Always wear appropriate personal protective equipment (PPE) when handling clinical specimen. e.g. lab coats, gloves, and eye protection.
4.1.14 The use of the DTPM COVID-10 RT-PCR test and data evaluation is restricted to trained laboratory personnel only.
4.1.15 Good laboratory practices (GLP) must be followed at all times to ensure the performance of assay components and the safety of laboratory personnel.
4.1.16 Refer to Centers for Disease Control and Prevention guidelines for important information regarding handling and testing for clinical

specimen from Patients Under Investigation for 2019 Novel Coronavirus (SARS-CoV-2).

4.1.17 Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where reagents and/or clinical specimen are handled.

4.1.18 Dispose of unused reagents and clinical specimen according to local, state, and federal regulations.

5.0 Sample Preparation and Nucleic Acid Extraction

5.1 Preparation of Patient Samples – Extraction of Nucleic Acids.

- Appropriate performance of PCR reactions requires careful sample handling and preparation to ensure a sufficient quantity of high-quality RNA template.
- Thaw frozen samples prior to use.
- The following nucleic acid extraction protocols are validated for use with the DTPM COVID-19 RT-PCR test.

Procedure A. Extract nucleic acids using a QIAGEN QIAmp Viral RNA Kit

Prepare reconstituted carrier RNA from lyophilized stock provided.

1. Add 310 µL buffer AVE to 310 µg of lyophilized Carrier RNA. Vortex 5-10 seconds. (1 µL/µg). Store unused material at -20ºC to -70ºC up to 6 months. Do not perform >3 freeze-thaw cycles of prepared materials.

Prepare Lysis Buffer containing carrier RNA.

2. Add 5.6 µL of reconstituted carrier RNA to 560 µL of Buffer AVL to process one sample in subsequent steps. Volumes may be made to scale based upon the number of samples to extract. e.g. 10 samples = [10 x 5.6 µL = 56 µL reconstituted RNA + 10 x 560 µL = 5.6 mL Buffer AVL] Store unused materials at 2ºC to 8ºC up to 72 hours.

a. Combine 200 µL patient sample and 500 µL Lysis Buffer containing carrier RNA into a 1.5 - 2 mL microcentrifuge tube. Close cap and vortex 5-10 seconds.

b. Incubate at room temperature (20-25C) for 10 minutes.

c. Add 600 µL (95 - 100%) ethanol. Close the cap and vortex 5-10 seconds.

d. Transfer 650 µL of lysate into Viral RNA spin column [housed in 2 mL collection tube]. Close cap.

e. Centrifuge at 8000 rpm for 60 seconds. Discard the filtrate.

f. Add the remaining 650 µL of lysate into spin column. Centrifuge at 8000 rpm for 60 seconds.

g. Transfer the Viral RNA spin column into a clean 2 mL collection tube.

h. Add 500 µL Buffer AW1 into Viral RNA spin column and centrifuge at 8000 rpm for 60 seconds.

i. Discard the filtrate or transfer the Viral RNA spin column into a clean 2 mL collection tube.

j. Add 500 µL Buffer AW2 into Viral RNA spin column and centrifuge at 8000 rpm for 3 minutes.

k. Transfer the Viral RNA spin column into a clean 1.5 - 2 mL capped collection tube.

l. Add 150 µL Buffer AVE or Nuclease Free water into Viral RNA spin column. Allow buffer AVE/water to rest 45 - 60 seconds and centrifuge at 8000 rpm for 60 seconds.

m. Dispose of the spin column. The filtrate is used as the “patient sample” in subsequent procedures.

Procedure B. Extract nucleic acids using a QIAGEN QIAamp 96 Viral RNA Kit

Prepare reconstituted carrier RNA from lyophilized stock provided.

1. Add 1550 µL buffer AVE to 1550 µg of lyophilized Carrier RNA. Vortex 5-10 seconds. (1 µL/µg). Store unused material at -20ºC to -70ºC up to 6 months. Do not perform >3 freeze-thaw cycles of prepared materials.

Prepare Lysis Buffer containing carrier RNA.

2. Add 5.6 µL of reconstituted carrier RNA to 560 µL of Buffer AVL to process one sample in subsequent steps. Volumes may be made to scale based upon the number of samples to extract. e.g. 10 samples = [10 x 5.6 µL = 56 µL reconstituted RNA + 10 x 560 µL = 5.6 mL Buffer AVL]. Invert container 8 - 10 times to mix. Store unused materials at 2ºC to 8ºC up to 72 hours.

a. Pipette 200 µL patient sample into separate wells using a 2.2 mL 96-well deep well plate.

b. Pipette 500 µL Lysis Buffer containing carrier RNA into each well. Mix the sample and Lysis Buffer by pipetting up and down 5 times.

c. Incubate at room temperature (20 - 25C) for 10 minutes.

d. Add 600 µL (95 - 100%) ethanol into each well. Mix thoroughly by pipetting up and down 5 times.

e. Place QIAamp 96 Plate onto a new 2.2 mL 96-well deep well plate.

f. Transfer 650 µL of lysate from step d into each well of the QIAamp 96 plate. Seal with an Airpore Tape sheet.

g. Load the QIAamp 96 Plate and 96-well deep well plate into the carrier, place carrier into the rotor bucket, and centrifuge at 6000 RPM for 4 minutes. Following centrifugation, if any sample has not passed through the membrane, spin for an additional 1 - 2 minutes.

h. Carefully remove the Airpore Tape sheet. Repeat steps f and g using the remaining lysate. Discard the 2.2 mL 96-well deep well plate containing the filtrate.

i. Place the QIAamp 96 Plate on a new 2.2 mL 96-well deep well plate. Carefully remove the Airpore Tape sheet. Add 500 µL Buffer AW1 into each well. Seal the QIAamp 96 plate with a new Airpore Tape sheet. Load QIAamp 96 plate and 2.2 mL 96-well deep well plate into the carrier, place carrier into the rotor bucket, and centrifuge at 6000 RPM for 4 minutes.

j. Carefully remove the Airpore Tape sheet and add 500 µL Buffer AW2 into each well. Seal the QIAamp 96 plate with a new Airpore Tape sheet. Load QIAamp 96 plate and 2.2 mL 96-well deep well plate into the carrier, place carrier into the rotor bucket, and centrifuge at 6000 RPM for 4 minutes.

k. Remove the Airpore Tape sheet and add 250 µL (95 - 100%) ethanol into each well. Seal the QIAamp 96 plate with a new Airpore Tape sheet. Load QIAamp 96 plate and 2.2 mL 96-well deep well plate into the carrier, place carrier into the rotor bucket, and centrifuge at 6000 RPM for 4 minutes. Discard the 2.2 mL 96-well deep well plate containing the filtrate.

l. Place the QIAamp 96 Plate on a new 2.2 mL 96-well deep well plate. Remove the Airpore Tape sheet. Load QIAamp 96 plate and 2.2 mL 96-well deep well plate into the carrier, place carrier into the rotor bucket, and centrifuge at 6000 RPM (without an Airpore Tape sheet) for 5 minutes to dry the membrane. Discard the 2.2 mL 96-well deep well plate.

m. Place the QIAamp 96 Plate on a new 96-well elution plate*. Add 150 µL Buffer AVE or nuclease free water into each well. Seal the QIAamp 96 plate using a new Airpore Tape sheet. Allow to incubate at room temperature (20 – 25C) for 1 minute.

n. Load QIAamp 96 plate and elution plate into the carrier, place carrier into the rotor bucket, and centrifuge at 6000 RPM for 4 minutes.

o. Dispose of the QIAamp 96 plate. Seal the elution plate containing the filtrate which is used as the “patient sample” in subsequent procedures.

*If elution plate is not available, substitute a 1.0 mL or 2.2 mL 96-well deep well plate.

Procedure C. Extract nucleic acids using Indical Biosciences IndiMag Pathogen kit using King Fisher Flex Purification System

a. Equilibrate samples at room temperature (15-25°C).

b. If the volume of the sample is less than 200 µL, add PBS or 0.9% NaCl to a final volume of 200 µL.

c. Prepare the Buffer VXL mixture according to Table 1, for use in **step g** of the procedure. Prepare reagent materials used to scale (1 – 96 samples).

d. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for ~ 3 minutes before using for the first time or ~ 1 minute before subsequent uses.

Important: Do not add Proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates. Follow the procedure as described below (pipetting Proteinase K into the wells, followed by sample and then Buffer VXL mixture).

Table 1: Buffer VXL mixture preparation

Reagent	**Number of samples ***		
	1	48	96
Buffer VXL	100 µL	4.8 mL	9.6 mL
Buffer ACB	400 µL	19.2 mL	38.4 mL
MagAttract Suspension G	25 µL	1.2 mL	2.4 mL
Carrier RNA (1 µg/µL)	1 µL	48 µL	96 µL

* The volume prepared is 105% of the required volume to compensate for pipetting error and possible evaporation. Excess buffer should be discarded.

e. Label and prepare 4 x 96-well deep well plates (S-Block) and 1 x 96-well microplate (slots 2-6) according to Table 2.

Table 2. Instrument Setup and Reagent Volumes

Slot	Loading Message	Format	Item to add	Volume per Well (uL)
6	Load Rod Cover	96-well Deep well plate	Cover for 96-tip comb	-
5	Load Elution	96-well micro plate	Buffer AVE	150
4	Load Wash 3	96-well Deep well plate	Ethanol (96-100%)	750
3	Load Wash 2	96-well Deep well plate	Buffer AW2	700
2	Load Wash 1	96-well Deep well plate	Buffer AW1	700
1	Load Lysate	96-well Deep well plate	Lysate*	720
	* Include 20 uL Proteinase K, 200 uL sample, and 500 uL Buffer VXL mixture			

f. Ensure to have prepared enough Buffer VXL mixture according to Table 1.

g. Pipette 20 µL Proteinase K into the bottom of a new well of the 96-well deep well plate or S-Block and add 200 µL sample.

Note: If your sample volume is less than 200 µL, bring it to 200 µL by adding PBS. Mix Buffer VXL mixture thoroughly for 30 s and add 500 µL Buffer VXL mixture to each sample in the 96-well deep well plate.

h. Immediately load the prepared plates onto the processor and start the protocol (IndiMag_Pathogen_KF_Flex.bdz).

i. Upon completion of the script [**step h**] remove the 96-well micro plate containing the purified nucleic acids. This plate contains the sample used for analysis. Samples may be pipetted directly from the plate or individually transferred into capped vials for further use and/or storage.

Procedure D. Extract nucleic acids using Omega Mag-Bind Viral DNA/RNA 96 kit using King Fisher Flex Purification System

Prepare Master Mixes

[Refer to manufacturer's package insert for preparation and storage of kit components]

1. Prepare the lysis master mix according to table below. Prepare fresh before each extraction run.

Buffer	Volume (µL)	Total Volume (96 samples)
TNA Lysis Buffer	240	25.4 mL*
Carrier RNA	1	105 µL*
*includes approximately 10% overage to account for error.		

2. Prepare the binding master mix according to table below. Prepare fresh before each extraction run.

Buffer	Volume (µL)	Total Volume (96 samples)
100% Isopropanol	280	29.6 mL*
Mag-Bind Particles RQ	5	530 µL*
*includes approximately 10% overage to account for error.		

Protocol for Respiratory Samples in Transport Media:

a. Transfer 200 µL respiratory sample in transport media or PBS to each well of a 96 deep-well plate. **Note:** If your sample volume is less than 200 µL, bring it to 200 µL by adding PBS.

b. Add 241 µL lysis master mix to each sample. Mix samples by pipetting up and down 10 times.

c. Add 285 µL binding master mix to each sample. Mix samples by pipetting up and down 10 times.

d. Prepare other reagent plates according to Plate Layout table below.

e. Start program on KingFisher Flex (Omega_M6219_150Elution_KFF.bdz) and load plates onto instrument when prompted.

f. Upon completion of the script remove the 96-well plate containing the purified nucleic acids. This plate contains the sample used for analysis. Samples may be pipetted directly from the plate or individually transferred into capped vials for further use and/or storage. Seal plate containing purified nucleic acids if not used within 5 minutes.

Plate Layout Table – KingFisher Flex Instrument Test Plate Configuration

Plate Position	Plate Type	Content (per well)	Volume (µL)
1	96 deep-well	Lysate (lysis master mix, sample, MBP CNR, & ProK Solution)	726
2	96 deep-well	RMP Wash Buffer	350
3	96 deep-well	80% Ethanol	350
4	96 deep-well	80% Ethanol	350
5	96 well	Nuclease-Free Water	150
6	96 deep-well	96 tip-comb for deep-well magnets	n/a
7	not used	not used	n/a
8	not used	not used	n/a

Procedure E. Extract nucleic acids using ThermoFisher MagMax Viral Pathogen II (MVP II) Nucleic Acid Isolation kit using King Fisher Flex Purification System

a. Equilibrate samples at room temperature (15-25°C).

b. If the volume of the sample is less than 200 µL, add PBS or 0.9% NaCl to a final volume of 200 µL.

c. Prepare the Binding Bead Mixture according to Table 1. Prepare reagent materials used to scale (1 – 96 samples).

d. Before adding Binding Beads, ensure that it is fully resuspended. Vortex for ~ 3 minutes before using for the first time or ~ 1 minute before subsequent uses.

Important: Do not add Proteinase K directly to the Binding Bead Mixture! This can cause clogs or precipitates.

Table 1: Binding Bead Mixture Preparation

Reagent	**Volume per well ***
Binding Solution	265 µL
Binding Beads	10 µL
Total Volume per well	275 µL

* The volume prepared is 110% of the required volume to compensate for pipetting error and possible evaporation. Excess buffer should be discarded.

e. Label and prepare 3 x 96-well deep well plates (S-Block) and 1 x 96-well microplate (slots 2-5) according to Table 2.

Table 2. Instrument Setup and Reagent Volumes

Plate ID	**Plate Type**	**Plate Position**	**Content**	**Volume (µL)**
Sample Plate	96 deep-well	1	Sample Lysis Plate	480
Wash 1	96 deep-well	2	Wash Solution	500
Wash 2	96 deep-well	3	80% Ethanol	500
Elution Plate	96 deep-well	4	Elution Buffer (or RNase Free Water)	150
Tip Comb Plate	96 deep-well	5	Tip Comb	N/A

f. Add 200 µL sample to each required well in the sample lysis plate.

g. Pipette 275 µL Binding Bead mixture into the bottom of each well (required) of the sample lysis plate.

h. Add 5 µL Proteinase K to each well required in the sample lysis plate.

i. Start the protocol (**MVP_2Wash_200_Flex**.bdz). Immediately load each of the prepared plates onto the KingFisher Flex following the instrument prompts.

j. Upon completion of the script [**step i**] remove the 96-well plate containing the purified nucleic acids. This plate contains the sample used for analysis. Samples may be pipetted directly from the plate or individually transferred into capped vials for further use and/or storage. Seal plate containing purified nucleic acids if not used within 5 minutes.

6.0 PCR Reaction Setup

- Ensure all work surfaces are properly decontaminated prior to handling reagents and clinical samples.
- Ensure all components of the DTPM RT-PCR test are thawed and ready for use.
- If necessary, ensure clinical samples are thawed and ready for use.
- Ensure sufficient assay reagents are available according to the sample batch size. Refer to the table in section 6.1 for preparation of assay components.

Each PCR reaction plate contains:

1.) **Test** (Fast Virus 1-Step Enzyme Master Mix, primers and probe for SARS-CoV-2 RNA, the endogenous (internal) control [DTPM COVID-19 RT-PCR Multiplex Assay Mix] and nuclear material isolated from a patient sample.

2.) **Positive Control** (Fast Virus 1-Step Enzyme Master Mix, primers and probe for SARS-CoV-2 RNA, the endogenous (internal) control [DTPM COVID-19 RT-PCR Multiplex Assay Mix] and each corresponding positive control template)

3.) **No Template Control** (Fast Virus 1-Step Enzyme Master Mix, primers and probe for SARS-CoV-2 RNA, the endogenous (internal) control [DTPM COVID-19 RT-PCR Multiplex Assay Mix], and molecular grade water [No template DNA or RNA is included].

6.1 PCR Reaction Assay Configuration

384-well plate configuration

Assay components may be prepared to scale according to sample batch size. Be sure include both positive and negative controls in the batch calculation.

Find the total prepared assay component volume by multiplying the volumes per reaction components by n+2 (“n” being the number of total samples including controls). When calculating the pipetting volumes for each component, please use the volume table below. For example, for 10 samples, + 1 positive control, + 1 negative control, the volumes of assay components should be multiplied by 14 (12 samples & controls, +2 additional). Combine DTPM COVID-19 RT-PCR Multiplex Assay Mix and Fast Virus 1-Step Enzyme Master Mix into a single container and mix thoroughly by pipette. Please use caution when pipette mixing to avoid creation of bubbles in the combined assay components.

Assay Component	Volume
COVID-19 RT-PCR Multiplex Assay Mix	0.75 µL
ThermoFisher Fast Virus 1-Step Enzyme Master Mix	1.25 µL
Isolated template from Sample/Control	3.0 µL
Total Reaction Volume	5.0 µL

For example, 0.75 µL x 14 samples = 10.5 µL
1.25 µL x 14 samples = 17.5 µL
Total assay component = 28 µL

6.2 PCR Reaction Plate Configuration

1. Distribute 2.0 µL of the combined assay components into each well of the 384-well PCR reaction plate as necessary for clinical samples and controls.
2. Pipette the No Template Control (3 µL Molecular Grade Water) into the No Template Control well.
3. Pipette 3 µL of each Patient Sample into the respective corresponding wells of the PCR reaction plate.
4. Pipette 3 µL of Positive Control mix into the Positive Control well.
5. Seal PCR reaction plate.
6. Transfer the PCR reaction plate a suitable PCR instrument.

Example 1. Pipette Scheme for preparation of 384-well PCR Reaction Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10														
B																								
C																								
D																								
E																								
F																								
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O																								
P	PC																							NTC

6.3 PCR Instrument Configuration

Step	Cycles	Temperature	Duration
Reverse Transcription	1	50°C	10 minutes
Taq Polymerase Activation	1	95°C	20 seconds
Amplification	40	95°C	3 seconds
		60°C	20 seconds

Cycle conditions should evaluate amplification during the 60°C extension step. Each target template reporter and quencher dyes are outlined below. ROX is the passive reference dye contained in the ThermoFisher 1-Step Fast Virus Enzyme mix and is used to normalize instrument fluorescence.

Target Template	Reporter Dye	Quencher Dye
SARS-CoV-2 RNA	FAM™	NFQ-MGB
Endogenous (Internal) Control	VIC	NFQ-MGB
Passive Reference Dye	ROX	N/A

7.0 Quality Controls

All Quality Controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

NTC (no template control): A “no template” (negative) control (NTC) is included in each run and taken through the full sample processing procedure starting with extraction. It is needed to monitor for potential contamination of reagents or samples with nucleic acid containing the target sequence and it should be added once for each PCR run. The NTC consists of molecular grade water and should be undetermined for all targets (no Ct value).

Positive control: The positive control is necessary to demonstrate the functionality of all enzymes and reagents within the test procedure. The positive control consists of synthetic template which represents a conserved region of the SARS-CoV-2 N gene. A positive control should be added once for each PCR run. A positive control result must have Ct value ≤35.

Endogenous internal control: The endogenous internal control evaluates the presence of ubiquitously expressed human ribosomal RNA which is expected to be present/detected within clinical samples. The endogenous control template is coextracted with other nucleic acid which may be present in the native sample and serves to demonstrate the efficacy of the extraction step. Each endogenous internal control must exceed the cutoff (Ct≤35) for each clinical specimen to report any result. In the case of a “not detected” endogenous control result, users are instructed to repeat sample preparation and analysis. If the endogenous control is not detected in replicate analysis or if insufficient sample material is available to repeat the sample analysis, the specimen should be reported as “Invalid.” Subsequent request for a new sample collection is recommended.

If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run, perform a root cause analysis and re-test after the root cause has been eliminated.

8.0 Result Interpretation

Examination and Interpretation of Patient Specimen Results:

The DTPM COVID-19 RT-PCR test result interpretation algorithm is described below. For quality controls and clinical samples to be positive (detected), the amplification must exceed the threshold and generate a Ct value ≤35. Any result Ct>35 or “undetermined” should be interpreted as not detected. Refer to the test limitations, Section 10.0.

Result interpretation algorithm is listed for each target template in the table below.

Table 1. DTPM COVID-19 RT-PCR Test Results Interpretation

SARS-CoV-2 N Gene	Endogenous Internal Control	Result Interpretation	Report
+ (Ct ≤35)	+ (Ct≤35)	SARS-CoV-2 Detected*	Reactive (Positive/Detected)
- (Ct>35)	+ (Ct ≤35)	SARS-CoV-2 Not Detected	Non-reactive (Not Detected)
Any Result	- (Ct>35)	Invalid Result	Invalid. Repeat sample preparation and analysis. If second test yields an invalid result, report Invalid.

* Additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecoviruses currently unknown to infect humans, for epidemiological purposes or clinical management.

9.0 Calculations

9.1 Analyzing Data

1.) Confirm the Ct for the Positive Control is acceptable.
2.) Confirm the Ct for the Negative Control is acceptable.

a. **Reactive** (Positive/Detected): Samples which contain sufficient concentration of template should demonstrate amplification exceeding the threshold and background at or below the established cutoff (Ct ≤ 35). Results observed meeting this criterion are reported as “Positive/Detected.”

b. **Non-reactive** (Not Detected): Samples which DO NOT contain SARS-CoV-2 RNA or that contain low levels of template should demonstrate amplification ABOVE the cutoff (Ct > 35) or no amplification (“undetermined”). Results observed meeting this criterion are reported as “Not Detected.”

c. **Invalid** (No Result Reported): Samples for which amplification is not observed to exceed the cutoff (Ct ≤ 35) for the endogenous control assay, will be interpreted as “Invalid.” Samples which are initially determined to be “Invalid” *may* undergo a second preparation and analysis in the event some error may have occurred. If the subsequent analysis returns a second “Invalid” result, the sample will be reported as “Invalid.”

10.0 Limitations

1. The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

2. Specimens must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

3. Extraction and amplification of nucleic acid from clinical specimens must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

4. The DTPM COVID-19 RT-PCR test can be used only with the specimens listed in the Intended Use statement. Other specimen types have not been evaluated and should not be tested with this assay.

5. Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the DTPM COVID-19 RT-PCR test. SARS-CoV is not known to be currently circulating in the human population, and therefore is highly unlikely to be present in patient specimens.

6. The instrument and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this IFU.

7. False-negative results may arise from:
   - Improper specimen collection
   - Degradation of the viral RNA during shipping/storage
   - Using unauthorized extraction or assay reagents
   - The presence of RT-PCR inhibitors
   - Mutation in the SARS-CoV-2 virus
   - Failure to follow instructions for use

8. False-positive results may arise from:
   - Cross-contamination during specimen handling or preparation
   - Cross-contamination between patient samples
   - Specimen mix-up
   - RNA contamination during product handling

9. As with any molecular test, mutations within the target regions of DTPM COVID-19 RT-PCR test could affect primer and/or probe binding resulting in failure to detect the presence of virus.

10. The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

11. Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision. Follow up testing should be performed according to the current CDC recommendations.

12. Detection of SARS-CoV-2 RNA indicates presence of viral RNA; however this does not confirm that SARS-CoV-2 is the causative agent of clinical symptoms. Nucleic acid may persist even after the virus is no longer viable.

13. Laboratories are required to report all results to the appropriate public health authorities.

This real-time qualitative PCR technology is widely regarded as the definitive technique for the detection of endogenous and exogenous targets in human body fluids. Using target-specific primers and probes eliminates most analytical interference. However, due to the nature of the matrix analyzed in this procedure, interfering substances may be introduced. The endogenous internal control is thus designed to serve as an indicator for potential interfering substances in any patient sample.

11.0 Conditions of Authorization, Authorized Laboratories

The DTPM COVID-19 RT-PCR test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas

To assist clinical laboratories using the DTPM COVID-19 RT-PCR test, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories* using your product must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical

specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and you (via email or phone: help@DTPM.com; (256) 845-1261) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in real time RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

G. Authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

* The letter of authorization refers to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

12.0 Analytical Data Performance Evaluation

Limit of Detection (LoD):

The LoD of the multiplex DTPM COVID-19 RT-PCR test was determined using quantified Genomic RNA from SARS-CoV-2, Isolate USA-WA1/2020 (NR-52285) as well as SARS-CoV-2, Isolate USA-WA1/2020, Heat Inactivated (NR-52286) both obtained from BEI Resources. A preliminary LoD was determined by testing serial dilutions of SARS-CoV-2 RNA (500 – 0.2 genomic copies/µL) spiked into pooled negative nasopharyngeal swab clinical matrix collected in molecular transport media (MTM) in triplicate. Samples were prepared using the QIAamp Viral RNA Kit and manual extraction method in accordance with the standard operating procedure for this method.

The LoD was verified by testing 20 additional extraction replicates consisting of pooled negative nasopharyngeal swab clinical matrix collected in molecular

transport media (MTM) spiked at the preliminary LoD concentration of 1 copies/µL (NR-52286) prior to nucleic acid extraction. Results for LoD confirmation (NR-52286) yielded 20/20 detected extraction replicates and observed mean Ct 32.43 and standard deviation 0.75. Results for LoD confirmation (NR-52285) yielded 20/20 detected extraction replicates and observed mean Ct 31.24 and standard deviation 1.36. The results from LoD study are summarized in Table 2.

Table 2. Summary of LoD Determination

Sample ID	Sample Matrix	Spiking concentration (copies/µL)	Mean Ct Target 1 N-Gene	SD Ct Target 1 N-Gene	Mean Ct IC S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity
SARS-CoV-2, Isolate USA-WA1/2020, Heat Inactivated							
NR-52286 500	NPS	500	24.09	0.19	26.61	0.15	3/3 100%
NR-52286 125	NPS	125	26.43	0.32	26.93	0.07	3/3 100%
NR-52286 25	NPS	25	28.55	0.07	26.91	0.05	3/3 100%
NR-52286 5	NPS	5	31.16	0.70	26.94	0.09	3/3 100%
NR-52286 1	NPS	1	33.33	1.02	26.99	0.06	3/3 100%
NR-52286 0.2	NPS	0.2	37.99	3.48	27.12	0.02	1/3 33%
LoD Confirmation Replicates							
NR-52286 1	NPS	1	32.43	0.75	26.57	0.49	20/20 100%
Genomic RNA from SARS-CoV-2, Isolate USA-WA1/2020							
NR-52285 500	NPS	500	20.51	3.64	26.84	0.10	3/3 100%
NR-52285 125	NPS	125	23.80	0.68	26.77	0.24	3/3 100%
NR-52285 25	NPS	25	27.76	0.20	27.17	0.11	3/3 100%
NR-52285 5	NPS	5	30.14	0.11	27.33	0.15	3/3 100%
NR-52285 1	NPS	1	32.22	0.34	27.21	0.14	3/3 100%
NR-52285 0.2	NPS	0.2	34.47	0.83	27.37	0.22	2/3 66%
LoD Confirmation Replicates							
NR-52285 1	NPS	1	31.24	1.36	26.65	0.61	20/20 100%

To improve the inclusivity of the multiplex DTPM COVID-19 RT-PCR test and reduce the risk of false-negative results for the detection of SARS-CoV-2, B.1.1.529 (Omicron) variant, a second reverse primer was introduced into the device design which compensates for the nucleocapsid 9 nucleotide deletion: N E31del, N R32del, N S33del. To demonstrate the efficacy of this change and further, that no adverse impact is observed, a new LoD bridging study was performed in parallel using negative NPS clinical matrix and spiked heat-inactivated virus (BEI-52286). Results from the parallel study and confirmation of the LoD suggest equivalent performance of the assay with the inclusion of the second reverse primer and are presented in Table 3.

Table 3. Results for DTPM COVID-19 RT-PCR test (MP) - Including New Omicron Primer and DTPM COVID-19 RT-PCR TEST

DTPM COVID-19 RT-PCR test (MP) - Including New Omicron Primer						
Sample Matrix	**Spiking concentration Copies/µL**	Mean Ct Target 1 N-Gene	SD Ct Target 1 N-Gene	Mean Ct IC S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity
NPS	500	24.01	0.16	26.82	0.13	5/5 100%
NPS	125	26.10	0.12	26.91	0.07	5/5 100%
NPS	25	28.56	0.56	27.04	0.37	5/5 100%
NPS	5	30.74	0.67	26.94	0.27	5/5 100%
NPS	1	33.23	0.29	26.68	0.30	5/5 100%
NPS	0.2	36.18	2.21	27.46	0.63	2/5 40%
NPS	1	33.57	0.77	27.63	0.13	20/20 100%
DTPM COVID-19 RT-PCR Test						
Sample Matrix	**Spiking concentration Copies/µL**	Mean Ct Target 1 N-Gene	SD Ct Target 1 N-Gene	Mean Ct IC S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity
NPS	500	24.23	0.20	26.89	0.25	5/5 100%
NPS	125	26.24	0.21	26.83	0.21	5/5 100%
NPS	25	28.81	0.42	27.05	0.17	5/5 100%
NPS	5	30.75	0.56	26.79	0.32	5/5 100%
NPS	1	33.82	0.66	26.95	0.11	5/5 100%
NPS	0.2	37.56	2.91	27.85	0.23	1/5 20%

Inclusivity:

The multiplex DTPM COVID-19 RT-PCR test is designed to detect up to an expressed 81 base pair (bp) region of the nucleocapsid phosphoprotein (N gene) in the SARS-CoV-2 genome. The translated amplicon spans up to amino acids 16 to 42.

In silico inclusivity was evaluated on December 14, 2021 using the basic local alignment search tool (BLAST) which compares primary biological sequence information to the International Nucleotide Sequence Database Collaboration [comprised of the DNA data Bank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at the National Center for Biological Information (NCBI)] as well as the NCBI SARS-CoV-2 reference sequence database from GenBank.

Each oligonucleotide was entered as an independent query sequence. Sequence results were evaluated for the 276,121 target sequences which maintained full coverage of all three oligonucleotide-binding regions (Fwd, Rev primers, and Probe). Results demonstrated >99.5% sequence homology for the sequences evaluated (see Table 4 below).

Table 4. *In silico* analysis Summary

	N Gene	N Gene	N Gene	N Gene
	SARS-CoV-2 Fwd Primer (5'-3')	SARS-CoV-2 Rev Primer (5'-3')	Sars-CoV-2 v2.0 Om Rev	SARS-CoV-2 Probe (5'-3')
Oligonucleotide length (bp)	20	19	19	16
Total strains Evaluated	276,121	276,121	276,121	276,121
100% match	>99.5 %	>99.9 %	100%	100%
*mismatches < 0.0007 % of 276,121 sequences examined.				

In silico analysis was also performed against current variants of concern (VOC) and variants being monitored (VBM) as defined by the Centers for Disease Control and Prevention (CDC).

Variants of Concern (VOC):

As of December 14, 2021, Delta (B.1.617.2, AY.1, AY.2, AY.3) and Omicron (B.1.1.529) variants circulating in the United States are classified as a variant of concern. A database was constructed for the VOC and mismatches were evaluated for each component of the multiplex DTPM COVID-19 RT-PCR test (Fwd, Rev primers, and Probe) by multiple sequence alignment using ClustalW2. The *in silico* analysis demonstrated 100% sequence homology for the delta variant and omicron variant (using second reverse primer) examined in the binding region of the primers and probe. This indicates the VOC examined would be detected by the multiplex DTPM COVID-19 RT-PCR test.

		SARS-CoV-2 Fwd	SARS-CoV-2 Rev	SARS-CoV-2 v2.0 Om Rev	SARS-CoV-2 Probe
Variant of Concern (VOC)	Total VOC Examined	Homology	Homology	Homology	Homology
Delta (B.1.617.2, AY.1, AY.2, AY.3)	200	100%	100%	100%	100%
Omicron (B.1.1.529)	200	100%	47%	100%	100%

Variants Being Monitored (VBM):

As of December 14, 2021, there are no SARS-CoV-2 mutations indicated as a variant of interest. However, the following SARS-CoV-2 variants are indicated as a variant being monitored per the current CDC classifications: Alpha (B.1.1.7 and

Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2). The *in silico* analysis demonstrated 100% sequence homology for each variant examined in the binding region of the primers and probe. This indicates the VBM examined would be detected by the multiplex DTPM COVID-19 RT-PCR test.

In silico molecular analysis demonstrates that the region in the N gene used to design the multiplex DTPM COVID-19 RT-PCR test is highly conserved. Combined mismatches for the Fwd, Rev primers, and Probe remain below 0.05% of total sequences examined. Further, current VOC and VBM contain base pair substitutions that are not located within the conserved binding region of the primers or probe used in the multiplex DTPM COVID-19 RT-PCR test. Taken collectively, these analyses demonstrate that all the SARS-CoV-2 variants examined are expected to be detected by the multiplex DTPM COVID-19 RT-PCR test.

		SARS-CoV-2 Fwd	SARS-CoV-2 Rev	SARS-CoV-2 v2.0 Om Rev	SARS-CoV-2 Probe
Variant Being Monitored	Total VBM Examined	Homology	Homology	Homology	Homology
Alpha (B.1.1.7, Q.x lineages)	20	100%	100%	100%	100%
Beta (B.1.351, B.1.351.2, B.1.351.3)	9	100%	100%	100%	100%
Gamma (P.1, P.1.1, P.1.2)	13	100%	100%	100%	100%
Epsilon (B.1.427, B.1.429)	13	100%	100%	100%	100%
Eta (B.1.525)	9	100%	100%	100%	100%
Iota (B.1.526)	20	100%	100%	100%	100%
Kappa (B.1.617.1)	20	100%	100%	100%	100%
1.617.3	13	100%	100%	100%	100%
Mu (B.1.621, B.1.621.1)	20	100%	100%	100%	100%
Zeta (P.2)	20	100%	100%	100%	100%

Specificity to SARS-CoV-2 B.1.1.529 [Omicron] variant

To demonstrate empirically the multiplex DTPM COVID-19 RT-PCR test reactivity against the omicron variant, a synthetic construct was synthesized and analyzed in parallel. *In silico* analysis suggested the design of the single reverse primer would not bind to the B.1.1.529 9nt deletion mutation in the nucleocapsid protein. To mitigate the risk of false-negative results, the addition of the second reverse primer was introduced into the device design which compensates for the 9nt deletion. Parallel analysis using the omicron variant positive control as the representative template demonstrates the failure of the existing reverse primer to bind, as anticipated. However, the addition of the second reverse primer is demonstrated to be an effective resolution to the non-reactivity observed in the single reverse design. Serial dilution samples were prepared in molecular grade water and exposed to both assay configurations. Results from the comparison are present in Table 5.

Table 5. Comparison of samples containing Omicron Positive Control Plasmid tested on DTPM COVID-19 RT-PCR test compared to assay including new Omicron primer

DTPM COVID-19 RT-PCR test (MP) - Including New Omicron Primer			
Sample ID	**Spiking concentration Copies/µL**	**Mean Ct Target 1 N-Gene**	**Reactivity**
SARS-CoV-2, Omicron Positive Control Plasmid with 9nt deletion			
1 x10^3	1 x10^3	29.41	3/3 100%
1 x10^2	1 x10^2	32.34	3/3 100%
1 x10^1	1 x10^1	33.86	3/3 100%
NTC	0	Undetermined	Not Detected
DTPM COVID-19 RT-PCR test (MP) - Current Device			
SARS-CoV-2, Omicron Positive Control Plasmid with 9nt deletion			
1 x10^3	1 x10^3	Undetermined	0/3 0%
1 x10^2	1 x10^2	Undetermined	0/3 0%
1 x10^1	1 x10^1	Undetermined	0/3 0%
NTC	0	Undetermined	Not Detected

Additional empirical analysis was performed using synthetic RNA constructs to challenge the reactivity of the DTPM COVID-19 RT-PCR Test for both Delta (B.1.617.2; Twist Biosciences – Control 23; Catalogue #104533) and Omicron (B.1.1.529; Twist Biosciences – Control 48; Catalogue #105204) variants of SARS-

CoV-2. Parallel dilution series were prepared in triplicate using negative NPS clinical matrix and template extracted using the Qiagen Viral RNA kit. Each dilution series was evaluated using both the single reverse primer design as well as the design which includes the second reverse primer. Results from these analyses suggest equivalent device performance for both designs exposed to the Delta (B.1.617.2) variant. Based upon observations from this study, there appears to be reduced sensitivity of the single reverse primer design when exposed to the Omicron (B.1.1.529) variant. However, the design which contains the second reverse primer demonstrates equivalent performance when exposed to either Delta or Omicron variants. Results from the comparison are present in Table 6.

Table 6. Comparison of contrived NPS samples tested on DTPM COVID-19 RT-PCR test compared to assay including new Omicron primer

			DTPM COVID-19 RT-PCR test (MP) - Including New Omicron Primer					DTPM COVID-19 RT-PCR test (MP)				
Sample ID	Sample Matrix	Spiking concentration (copies/uL)	Mean Ct Target 1 N-Gene*	SD Ct Target 1 N-Gene	Mean Ct IC S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity	Mean Ct Target 1 N-Gene*	SD Ct Target 1 N-Gene	Mean Ct IC S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity
Twist Biosciences Delta Variant Control 23 (Pango Lineage B.1.617.2)												
control 23 500 copies	NPS	500	24.60	0.36	27.12	0.24	3/3 100%	25.30	0.17	27.31	0.17	3/3 100%
control 23 125 copies	NPS	125	25.61	0.67	26.57	0.09	3/3 100%	26.96	0.35	27.18	0.18	3/3 100%
control 23 25 copies	NPS	25	28.12	0.88	27.00	0.40	3/3 100%	28.87	0.55	27.23	0.19	3/3 100%
control 23 1 copies	NPS	1	31.75	0.81	27.00	0.12	3/3 100%	32.63	0.53	27.18	0.18	3/3 100%
control 23 0.2 copies	NPS	0.2	32.65	0.22	26.74	0.16	2/3 66%	34.51	N/A	27.28	0.38	1/3 33%
Twist Biosciences Omicron Variant Control 48 (Pango Lineage B.1.1.529)												
control 48 500 copies	NPS	500	25.75	0.37	26.98	0.04	3/3 100%	33.57	0.05	27.28	0.02	2/3 66%
control 48 125 copies	NPS	125	27.99	0.17	27.20	0.12	3/3 100%	33.74	N/A	26.60	0.54	1/3 33%
control 48 25 copies	NPS	25	29.60	0.41	27.12	0.13	3/3 100%	N/A	N/A	27.35	0.11	0/3 0%
control 48 1 copies	NPS	1	34.03	0.80	27.44	0.05	3/3 100%	N/A	N/A	27.63	0.04	0/3 0%
control 48 0.2 copies	NPS	0.2	N/A	N/A	27.25	0.29	0/3 0%	N/A	N/A	27.24	0.07	0/3 0%

*Mean values were calculated for "Detected" results only
N/A- Not Applicable

To further demonstrate efficacy of the DTPM COVID-19 RT-PCR test, heat-inactivated SARS-CoV-2 (B.1.1.529) virus was obtained from BEI Resources (NR-56495 SARS-Related Coronavirus 2 Isolate hCoV-19/USA/GA-EHC-2811C/2021 (Lineage B.1.1.529; Omicron Variant), Heat Inactivated). Serial dilutions were prepared by spiking heat-inactivated B.1.1.529 variant into pooled negative nasopharyngeal swab clinical matrix collected in molecular transport media (MTM), in triplicate. Samples were purified using the Qiagen QIAamp Viral RNA (manual) extraction kit and analyzed in accordance with the standard operating procedure. Summarized results from the serial dilution series are presented below in Table 7.

Table 7. Summarized observations of serial dilution reactivity using heat-inactivated SARS-CoV-2 B.1.1.529 "Omicron" variant for the DTPM COVID-19 RT-PCR test

Sample ID	Sample Matrix	Sample Matrix Spiking Concentration (Copies/uL)	Mean Ct N-Gene	SD Ct N-Gene	Mean Ct S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity
NR-56495 500 copies	NPS	500	24.86	0.08	25.71	0.04	3/3
NR-56495 125 copies	NPS	125	26.42	0.20	25.71	0.14	3/3
NR-56495 25 copies	NPS	25	29.04	0.09	25.92	0.10	3/3
NR-56495 5 copies	NPS	5	31.46	0.18	25.12	0.03	3/3
NR-56495 1 copies	NPS	1	33.91	0.71	25.52	0.04	3/3
NR-56495 0.2 copies	NPS	0.2	N/A	N/A	25.77	0.16	0/3

N/A- Not Applicable

Analytical Specificity:

In silico analysis was performed to evaluate the potential for cross-reaction for the assay with the inclusion of the second reverse primer. No homology was observed for the organisms indicated in Table 8 below.

Table 8. Cross-reactivity Analysis

	In Silico Sequence Homology (%)[2]				
Pathogen	Accession #[1]	SARS-CoV-2 Fwd	SARS-CoV-2 Rev	SARS-CoV-2 v2.0 Om Rev	SARS-CoV-2 Probe
Adenovirus	AC_000019.1	None	None	None	None
B. pertussis	NC_018518	None	None	None	None

Bocavirus	NC_012729.2	None	None	None	None
C. pneumoniae	NC_000922.1	None	None	None	None
Coronavirus 229E	NC_002645.1	None	None	None	None
Coronavirus HKU-1	NC_006577.2	None	None	None	None
Coronavirus NL63	NC_005831.2	None	None	None	None
Coronavirus OC43	NC_006213.1	None	None	None	None
Enterovirus	NC_038308.1	None	None	None	None
H. influenzae	NZ_CP031681.1	None	None	None	None
Influenza A	NC_026426.1	None	None	None	None
Influenza B	NC_002204.1	None	None	None	None
K. pneumoniae	NZ_KE504629.1	None	None	None	None
Legionella	NC_009494.2	None	None	None	None
M. catarrhalis	CP018059.1	None	None	None	None
M. pneumoniae	CP039761.1	None	None	None	None
Metapneumovirus A	NC_39199.1	None	None	None	None
Parechovirus	NC_001897.1	None	None	None	None
Parainfluenza virus 1	NC_003461.1	None	None	None	None
Parainfluenza virus 2	NC_003443	None	None	None	None
Parainfluenza virus 3	NC_001796.2	None	None	None	None

Parainfluenza virus 4	NC_021928.1	None	None	None	None
Rhinovirus	NC_038311.1	None	None	None	None
Respiratory syncytial virus A	NC_038235.1	None	None	None	None
S. aureus	CP018629.1	None	None	None	None
S. pneumoniae	NZ_CP053210	None	None	None	None
Salmonella enterica Typhimurium	CP074092.1	None	None	None	None

[1] RefSeq defined by NCBI as comprehensive, integrated, non-redundant, curated anchor sequence.
[2] Sequence homology <80% identified as None.

In addition, each component of the assay (Forward, Reverses, and Probe sequences) was subjected to an *in silico* analysis to demonstrate specificity of the assay components against multiple whole genome sequences for additional organisms as detailed in Table 9 below.

Table 9. Cross-reactivity organisms evaluated *in silico*

	In Silico Sequence Homology (%)[2]				
Pathogen	Accession #[1]	SARS-CoV-2 Fwd	SARS-CoV-2 Rev	SARS-CoV-2 v2.0 Om Rev	SARS-CoV-2 Probe
SARS-CoV-1	NC_004718.3	90	89.5	74	87.5
MERS	NC_019843.3	None	None	None	None
Mycobacterium tuberculosis	NZ_CP0477163.1	None	None	None	None
Streptococcus pyogenes	CP041615.1	None	None	None	None
Pneumocystis jirovecii	NC_020331.1	None	None	None	None
Candida albicans	NC_032092.1 NC_032089.1 NC_032095.1	None	None	None	None
Pseudomonas aeruginosa	CP022001	None	None	None	None
Staphylococcus epidermidis	NZ_CP035288	None	None	None	None
Streptococcus salivarius	NZ_CP066093.1	None	None	None	None

[1] RefSeq defined by NCBI as comprehensive, integrated, non-redundant, curated anchor sequences.
[2] Sequence homology <80% identified as None

The DTPM primer and probe sequences showed homology to SARS-CoV-1. Further testing may be necessary to differentiate between SARS-CoV-1 or other Sarbecoviruses currently unknown to infect humans.

13.0 Clinical Performance Evaluation

Performance of the multiplex DTPM COVID-19 RT-PCR test was evaluated using 60 individual, nasopharyngeal (NP) swab retrospective specimen collected from individuals suspected of COVID-19 by a healthcare professional.

A population of archived samples were first evaluated using an FDA authorized molecular comparator assay. From this population, 30 negative and 30 positive samples were selected for comparison against the candidate test. Samples were selected which represented "detected" results across the viral load range, including "weak positive" challenging samples to represent at least 20% of the detected population as determined by the comparator assay.

Each comparator sample underwent comparator testing per authorized IFU. Each of the selected samples were subsequently tested for detection of SARS-CoV-2 RNA using Qiagen Viral RNA manual extraction and multiplex DTPM COVID-19 RT-PCR test workflow. Positive Percent Agreement was observed to be 96.7% (29/30). Negative Percent Agreement was observed to be 100% (30/30).

Table 10. Clinical Validation Summary of Results

	FDA authorized molecular comparator assay POSITIVE	FDA authorized molecular comparator assay NEGATIVE
DTPM COVID-19 RT-PCR TEST POSITIVE	29	0
DTPM COVID-19 RT-PCR TEST NEGATIVE	1	30

PPA: 96.7% (29/30), 95% CI: 83.3% - 99.4%
NPA: 100% (30/30), 95% CI: 88.7% - 100%

An additional clinical evaluation was performed to demonstrate the efficacy of the multiplex DTPM COVID-19 RT-PCR test including the second reverse primer. Residual frozen sample extracts from the previous clinical evaluation were thawed and analyzed in parallel using the multiplex DTPM COVID-19 RT-PCR test and the multiplex DTPM COVID-19 RT-PCR test containing the additional reverse primer. Comparative results for each sample are presented in Table 8.

Table 11. Concordance between re-tested frozen extract tested on DTPM COVID-19 RT-PCR test compared to assay including new Omicron primer

	DTPM COVID-19 RT-PCR test (MP) POSITIVE	DTPM COVID-19 RT-PCR test (MP) NEGATIVE
DTPM COVID-19 RT-PCR test (MP) – Including New Omicron Primer POSITIVE	27	1[b]
DTPM COVID-19 RT-PCR test (MP) – Including New Omicron Primer NEGATIVE	1[a]	31

[a)] This sample was positive by the DTPM COVID-19 RT-PCR test when tested in the original clinical validation and was positive by the comparator assay
[b)] This sample was positive by the DTPM COVID-19 RT-PCR test when tested in the original clinical validation and was positive by the comparator assay
PPA: 96.4 %, 95% CI: 82.3% - 99.4%
NPA: 96.9%. 95% CI: 84.3% - 99.5%

14.0 Bridge Data to support Alternative Nucleic Acid Extraction Kits for detection of SARS-CoV-2 RNA.

Additional nucleic acid purification methods were evaluated to establish efficacy for use with the multiplex DTPM COVID-19 RT-PCR test. Contrived samples were prepared using SARS-CoV-2, Isolate USA-WA1/2020, Heat Inactivated (NR-52286) obtained from BEI Resources spiked into pooled negative nasopharyngeal swab clinical matrix collected in molecular transport media (MTM). A series of five samples per concentration were prepared at 0.3x; 1x; and 3x LoD based upon the limit of detection established using the Qiagen QIAamp Viral RNA (manual) extraction kit. Results for each extraction material are outlined in the Table 12 below.

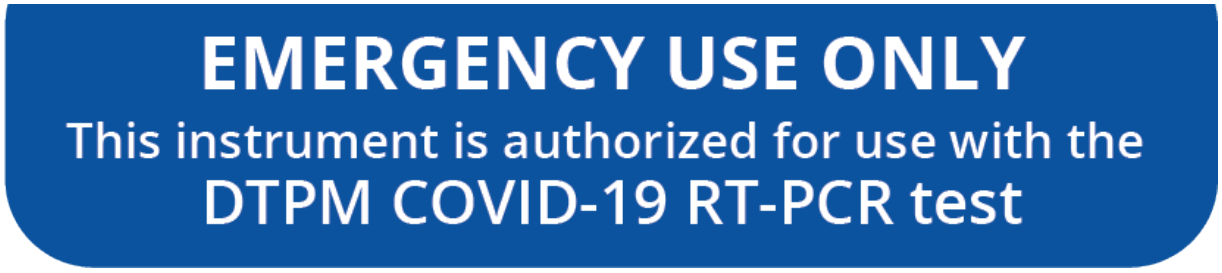
Table 12. Alternative Nucleic Acid Extraction Bridge Study Data

				DTPM COVID-19 RT-PCR test (MP)				
Sample ID	**Sample Matrix**	**Spiking concentration (copies/µL)**	**Replicate Number**	**Ct Target 1 N-Gene**	**Result Target 1 (based on cutoff)**	**Ct IC S9 ribosomal gene**	**Result IC (based on cutoff)**	**Final Result**
Indical Indimag Extraction Kit using King Fisher Flex								
Indi Mag 3x	NPS	3	1	33.02	Detected	28.92	Detected	Detected
Indi Mag 3x	NPS	3	2	32.79	Detected	28.93	Detected	Detected
Indi Mag 3x	NPS	3	3	32.73	Detected	29.14	Detected	Detected
Indi Mag 3x	NPS	3	4	32.44	Detected	28.92	Detected	Detected
Indi Mag 3x	NPS	3	5	31.97	Detected	28.85	Detected	Detected
Indi Mag 1x	NPS	1	1	Undetermined	Detected	28.45	Detected	Not Detected
Indi Mag 1x	NPS	1	2	34.29	Detected	28.60	Detected	Detected

Indi Mag 1x	NPS	1	3	33.74	Detected	28.44	Detected	Detected
Indi Mag 1x	NPS	1	4	34.12	Detected	28.42	Detected	Detected
Indi Mag 1x	NPS	1	5	33.24	Detected	28.28	Detected	Detected
Indi Mag 0.3x	NPS	0.3	1	Undetermined	Not Detected	28.31	Detected	Not Detected
Indi Mag 0.3x	NPS	0.3	2	Undetermined	Not Detected	27.96	Detected	Not Detected
Indi Mag 0.3x	NPS	0.3	3	33.21	Detected	28.09	Detected	Detected
Indi Mag 0.3x	NPS	0.3	4	Undetermined	Not Detected	28.03	Detected	Not Detected
Indi Mag 0.3x	NPS	0.3	5	Undetermined	Not Detected	28.06	Detected	Not Detected
Omega Mag-Bind Viral DNA/RNA 96 kit using King Fisher Flex								
Omega 3x	NPS	3	1	31.83	Detected	28.61	Detected	Detected
Omega 3x	NPS	3	2	32.10	Detected	28.39	Detected	Detected
Omega 3x	NPS	3	3	31.92	Detected	28.36	Detected	Detected
Omega 3x	NPS	3	4	31.62	Detected	28.38	Detected	Detected
Omega 3x	NPS	3	5	32.26	Detected	28.42	Detected	Detected
Omega 1x	NPS	1	1	32.76	Detected	28.38	Detected	Detected
Omega 1x	NPS	1	2	33.27	Detected	28.35	Detected	Detected
Omega 1x	NPS	1	3	33.82	Detected	28.41	Detected	Detected
Omega 1x	NPS	1	4	33.61	Detected	28.58	Detected	Detected
Omega 1x	NPS	1	5	33.57	Detected	28.37	Detected	Detected
Omega 0.3x	NPS	0.3	1	Undetermined	Not Detected	28.83	Detected	Not Detected
Omega 0.3x	NPS	0.3	2	Undetermined	Not Detected	28.90	Detected	Not Detected
Omega 0.3x	NPS	0.3	3	Undetermined	Not Detected	28.47	Detected	Not Detected
Omega 0.3x	NPS	0.3	4	Undetermined	Not Detected	28.92	Detected	Not Detected
Omega 0.3x	NPS	0.3	5	Undetermined	Not Detected	28.90	Detected	Not Detected
Qiagen QiaAmp96 Extraction Kit								
QiaAmp 96 52286 3x	NPS	3	1	28.76	Detected	26.73	Detected	Detected
QiaAmp 96 52286 3x	NPS	3	2	25.65	Detected	26.59	Detected	Detected
QiaAmp 96 52286 3x	NPS	3	3	30.61	Detected	27.36	Detected	Detected
QiaAmp 96 52286 3x	NPS	3	4	30.76	Detected	26.26	Detected	Detected
QiaAmp 96 52286 3x	NPS	3	5	25.28	Detected	26.26	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	1	32.53	Detected	27.42	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	2	34.45	Detected	27.38	Detected	Detected

QiaAmp 96 52286 1x	NPS	1	3	31.53	Detected	27.19	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	4	33.66	Detected	26.30	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	5	32.95	Detected	27.15	Detected	Detected
QiaAmp 96 52286 0.3x	NPS	0.3	1	34.66	Detected	26.92	Detected	Detected
QiaAmp 96 52286 0.3x	NPS	0.3	2	35.12	Not Detected	27.38	Detected	Not Detected
QiaAmp 96 52286 0.3x	NPS	0.3	3	34.53	Detected	27.11	Detected	Detected
QiaAmp 96 52286 0.3x	NPS	0.3	4	Undetermined	Not Detected	27.66	Detected	Not Detected
QiaAmp 96 52286 0.3x	NPS	0.3	5	19.30	Detected	27.63	Detected	Detected
MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using King Fisher Flex								
MagMax 3x	NPS	3	1	31.11	Detected	26.68	Detected	Detected
MagMax 3x	NPS	3	2	31.88	Detected	26.59	Detected	Detected
MagMax 3x	NPS	3	3	30.76	Detected	26.63	Detected	Detected
MagMax 3x	NPS	3	4	30.68	Detected	26.40	Detected	Detected
MagMax 3x	NPS	3	5	31.36	Detected	26.50	Detected	Detected
MagMax 1x	NPS	1	1	32.42	Detected	25.76	Detected	Detected
MagMax 1x	NPS	1	2	33.17	Detected	25.62	Detected	Detected
MagMax 1x	NPS	1	3	32.75	Detected	25.96	Detected	Detected
MagMax 1x	NPS	1	4	32.16	Detected	25.63	Detected	Detected
MagMax 1x	NPS	1	5	33.28	Detected	25.65	Detected	Detected
MagMax 0.3x	NPS	0.3	1	Undetermined	Not Detected	26.41	Detected	Not Detected
MagMax 0.3x	NPS	0.3	2	34.20	Detected	26.16	Detected	Detected
MagMax 0.3x	NPS	0.3	3	36.16	Not Detected	25.56	Detected	Not Detected
MagMax 0.3x	NPS	0.3	4	Undetermined	Not Detected	25.20	Detected	Not Detected
MagMax 0.3x	NPS	0.3	5	33.71	Detected	25.48	Detected	Detected

Emergency Use Only Label. Please Affix The Following Label In A Prominent Location To Any ThermoFisher QuantStudio 5 RT-PCR Instrument Used In Conjunction With The DTPM COVID-19 RT-PCR Test. The Label Must Remain In Place For The Duration Of Emergency Use Authorization.



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